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(54) Title: COMPOSITIONS AND METHODS FOR THE TREATMENT OF IMMUNE RELATED DISEASES

(57) Abstract: The present invention relates to compositions containing novel proteins and methods of using those compositions for the diagnosis and treatment of immune related diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a nucleotide sequence (SEQ ID NO:1) of a native sequence PRO1356 cDNA, wherein SEQ ID NO:1 is a clone designated herein as "DNA64886-1601".

5 Figure 2 shows the amino acid sequence (SEQ ID NO:2) derived from the coding sequence of SEQ ID NO:1 shown in Figure 1.

Figure 3 shows a nucleotide sequence (SEQ ID NO:3) of a native sequence PRO1268 cDNA, wherein SEQ ID NO:3 is a clone designated herein as "DNA64903-1553".

10 Figure 4 shows the amino acid sequence (SEQ ID NO:4) derived from the coding sequence of SEQ ID NO:3 shown in Figure 3.

Figure 5 shows a nucleotide sequence (SEQ ID NO:5) of a native sequence PRO1884 cDNA, wherein SEQ ID NO:5 is a clone designated herein as "DNA84318-2520".

Figure 6 shows the amino acid sequence (SEQ ID NO:6) derived from the coding sequence of SEQ ID NO:5 shown in Figure 5.

15 Figure 7 shows a nucleotide sequence (SEQ ID NO:7) of a native sequence PRO3444 cDNA, wherein SEQ ID NO:7 is a clone designated herein as "DNA87997".

Figure 8 shows the amino acid sequence (SEQ ID NO:8) derived from the coding sequence of SEQ ID NO:7 shown in Figure 7.

20 Figure 9 shows a nucleotide sequence (SEQ ID NO:9) of a native sequence PRO3151 cDNA, wherein SEQ ID NO:9 is a clone designated herein as "DNA89273".

Figure 10 shows the amino acid sequence (SEQ ID NO:10) derived from the coding sequence of SEQ ID NO:9 shown in Figure 9.

Figure 11 shows a nucleotide sequence (SEQ ID NO:11) of a native sequence PRO4322 cDNA, wherein SEQ ID NO:11 is a clone designated herein as "DNA92223-2567".

25 Figure 12 shows the amino acid sequence (SEQ ID NO:12) derived from the coding sequence of SEQ ID NO:11 shown in Figure 11.

Figure 13 shows a nucleotide sequence (SEQ ID NO:13) of a native sequence PRO9964 cDNA, wherein SEQ ID NO:13 is a clone designated herein as "DNA96973".

30 Figure 14 shows the amino acid sequence (SEQ ID NO:14) derived from the coding sequence of SEQ ID NO:13 shown in Figure 13.

Figure 15 shows a nucleotide sequence (SEQ ID NO:15) of a native sequence PRO10008 cDNA, wherein SEQ ID NO:15 is a clone designated herein as "DNA101921".

Figure 16 shows the amino acid sequence (SEQ ID NO:16) derived from the coding sequence of SEQ ID NO:15 shown in Figure 15.

35 Figure 17 shows a nucleotide sequence (SEQ ID NO:17) of a native sequence PRO19598 cDNA, wherein SEQ ID NO:17 is a clone designated herein as "DNA145887".

Figure 18 shows the amino acid sequence (SEQ ID NO:18) derived from the coding sequence of SEQ ID NO:17 shown in Figure 17.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I.

Definitions

When a positive colony was isolated, a portion of it was picked by a toothpick and diluted into sterile water (30 μ l) in a 96 well plate. At this time, the positive colonies were either frozen and stored for subsequent analysis or immediately amplified. An aliquot of cells (5 μ l) was used as a template for the PCR reaction in a 25 μ l volume containing: 0.5 μ l Klentaq (Clontech, Palo Alto, CA); 4.0 μ l 10 mM dNTP's (Perkin Elmer-Cetus); 2.5 μ l Klentaq buffer (Clontech); 0.25 μ l forward primer; 0.25 μ l reverse primer; 12.5 μ l distilled water. When these clones were sequenced and when a significant homology was found, this resulted in the identification of additional EST sequences which either corresponded to full-length clones, which were examined and sequenced or served as a template for the creation of cloning oligonucleotides which were then used to screen various tissue libraries resulting in isolation of DNA encoding a native sequence PRO polypeptide.

EXAMPLE 6: Isolation of cDNA Clones Encoding Human PRO Polypeptides

Using the techniques described in Examples 1 to 5 above, numerous full-length cDNA clones were identified as encoding PRO polypeptides as disclosed herein. These cDNAs were then deposited under the terms of the Budapest Treaty with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC) as shown in Table 7 below. DNA87997, DNA89273, DNA96973, and DNA101921 were not deposited with the ATCC, but can be easily found using Genbank accession numbers HUMAPOD, HSLHRH1, HSAF001620, and HUMMIP1A respectively.

20

Table 7

<u>Material</u>	<u>UNQ</u>	<u>PRO</u>	<u>ATCC #</u>	<u>ATCC Deposit Date</u>
DNA64886-1601	705	1356	203241	September 9, 1998
DNA64903-1553	655	1286	203223	September 15, 1998
DNA84318-2520	867	1884	203580	January 12, 1999
25 DNA87997	1452	3444	N/A	N/A
DNA89273	1486	3151	N/A	N/A
DNA92223-2567	1879	4322	203851	March 16, 1999
DNA96973	998	9964	N/A	N/A
DNA101921	887	10008	N/A	N/A
30 DNA145887	5793	19598	PTA-1532	March 21, 2000

*N/A not available.

EXAMPLE 7: Stimulatory Activity in Mixed Lymphocyte Reaction (MLR) Assay (no.24)

This example shows that the polypeptides of the invention are active as stimulators of the proliferation of T-lymphocytes. Compounds which stimulate proliferation of lymphocytes are useful therapeutically where enhancement of an immune response is beneficial. A therapeutic agent may also take the form of antagonists of the PRO polypeptides of the invention, for example, murine-human chimeric, humanized or human antibodies against the polypeptide, which would be expected to inhibit T-lymphocyte proliferation.

The basic protocol for this assay is described in *Current Protocols in Immunology*, unit 3.12; edited by J. E. Coligan, A. M. Kruisbeek, D. H. Marglies, E. M. Shevach, W. Strober, National Institutes of Health, Published by John Wiley & Sons, Inc.

More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis (one donor will supply stimulator PBMCs, the other donor will supply responder PBMCs). If desired, the cells are frozen in fetal bovine serum and DMSO after isolation. Frozen cells may be thawed overnight in assay media (37°C, 5% CO₂) and then washed and resuspended to 3 x 10⁶ cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate).

The stimulator PBMCs are prepared by irradiating the cells (about 3000 Rads). The assay is prepared by plating in triplicate wells a mixture of: 100 µl of test sample diluted to 1% or to 0.1%; 50 µl of irradiated stimulator cells and 50 µl of responder PBMC cells. 100 microliters of cell culture media or 100 microliter of CD4-IgG is used as the control. The wells are then incubated at 37°C, 5% CO₂ for 4 days. On day 5 and each well is pulsed with tritiated thymidine (1.0 mCi/well; Amersham). After 6 hours the cells are washed 3 times and then the uptake of the label is evaluated.

In another variant of this assay, PBMCs are isolated from the spleens of Balb/c mice and C57B6 mice. The cells are teased from freshly harvested spleens in assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate) and the PBMCs are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging at 2000 rpm for 20 minutes, collecting and washing the mononuclear cell layer in assay media and resuspending the cells to 1 x 10⁷ cells/ml of assay media. The assay is then conducted as described above.

The results of this assay for compounds of the invention are shown below in Table 8. Positive increases over control are considered positive with increases of greater than or equal to 180% being preferred. However, any value greater than control indicates a stimulatory effect for the test protein.

Table 8

<u>PRO</u>	<u>PRO Concentration</u>	<u>Percent Increase Over Control</u>
PRO3151	1.34 µM	30.2

EXAMPLE 8: Skin Vascular Permeability Assay (no.51)

This assay shows that certain PRO polypeptides stimulate an immune response and induce inflammation by inducing mononuclear cell, eosinophil and PMN infiltration at the site of injection of the animal. This skin vascular permeability assay is conducted as follows. Hairless guinea pigs weighing 350 grams or more are anesthetized with ketamine (75-80 mg/Kg) and 5 mg/Kg Xylazine intramuscularly (IM). A sample of purified PRO polypeptide or a conditioned media test sample is injected intradermally onto the backs of the test animals with 100 µL per injection site. It is possible to have about 10-30, preferably about 16-24, injection sites per animal. One mL of Evans blue dye (1% in physiologic buffered saline) is injected

intracardially. Blemishes at the injection sites are then measured (mm diameter) at 1hr, 6 hrs and 24 hrs post injection. Animals were sacrificed at the appropriate time after injection. Each skin injection site is biopsied and fixed in paraformaldehyde. The skins are then prepared for histopathologic evaluation. At least a minimal perivascular infiltrate at the injection site is scored as positive, no infiltrate at the site of injection is scored as negative. Results are given in Table 9.

Table 9

<u>PRO</u>	<u>Time (hrs)</u>	<u>Infiltrate</u>
PRO9964	1.00	positive
PRO9964	6.00	positive
PRO10008	1.00	positive
PRO10008	6.00	positive
PRO19598	1.00	positive
PRO19598	6.00	positive

EXAMPLE 9: Inhibitory Activity in Mixed Lymphocyte Reaction (MLR) Assay (no. 67)

This example shows that one or more of the PRO polypeptides are active as inhibitors of the proliferation of stimulated T-lymphocytes. Compounds which inhibit proliferation of lymphocytes are useful therapeutically where suppression of an immune response is beneficial.

The basic protocol for this assay is described in *Current Protocols in Immunology*, unit 3.12; edited by J. E. Coligan, A. M. Kruisbeek, D. H. Marglies, E. M. Shevach, W. Strober, National Institutes of Health, Published by John Wiley & Sons, Inc.

More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis (one donor will supply stimulator PBMCs, the other donor will supply responder PBMCs). If desired, the cells are frozen in fetal bovine serum and DMSO after isolation. Frozen cells may be thawed overnight in assay media (37°C, 5% CO₂) and then washed and resuspended to 3x10⁶ cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate). The stimulator PBMCs are prepared by irradiating the cells (about 3000 Rads).

The assay is prepared by plating in triplicate wells a mixture of:

100:1 of test sample diluted to 1% or to 0.1%,

50:1 of irradiated stimulator cells, and

50:1 of responder PBMC cells.

100 microliters of cell culture media or 100 microliter of CD4-IgG is used as the control. The wells are then incubated at 37°C, 5% CO₂ for 4 days. On day 5, each well is pulsed with tritiated thymidine (1.0 mCi/well; Amersham). After 6 hours the cells are washed 3 times and then the uptake of the label is evaluated.

In another variant of this assay, PBMCs are isolated from the spleens of Balb/c mice and C57B6 mice. The cells are teased from freshly harvested spleens in assay media (RPMI; 10% fetal bovine serum,

17/18FIGURE 17

CAGTTTCCTTCATCTGTAAACATCAAATGAATAATAATACCAATCTCCTAGACTTCATAAGA
GGATTAAACAAAGACAAAATATGGGAAAAACATAACATGGCGTCCCATTAATTATAGATCT
TATTATTGACACTAAAATGGCATTTAAATTTACCAAAGGAAGACAGCATCTGTTTCCTCT
TTGGTCCTGAGCTGGTTAAAAGGAACACTGGTTGCCTGAACAGTCACACTTGCAACCATG
ATGCCTAAACATTGCTTTCTAGGCTTCCTCATCAGTTTCTTCCTTACTGGTGTAGCAGGA
ACTCAGTCAACGCATGAGTCTCTGAAGCCTCAGAGGGTACAATTTCAGTCCCGAAATTTT
CACAAATTTTGCAATGGCAGCCTGGGAGGGCACTTACTGGCAACAGCAGTGTCTATTTT
GTGCAGTACAAAATCATGTTCTCATGCAGCATGAAAAGCTCTCACCAGAAGCCAAGTGGA
TGCTGGCAGCACATTTCTTGTAACCTCCAGGCTGCAGAACATTGGCTAAATATGGACAG
AGACAATGGAAAAATAAAGAAGACTGTTGGGGTACTCAAGAACTCTCTTGAGACCTTACC
AGTGAAACCTCAGACATACAGGAACCTTATTACGGGAGGGTGAGGGCGGCCTCGGCTGGG
AGCTACTCAGAATGGAGCATGACGCCGCGGTTCACTCCCTGGTGGGAAACAAAATAGAT
CCTCCAGTCATGAATATAACCCAAGTCAATGGCTCTTTGTTGGTAATTCTCCATGCTCCA
AATTTACCATATAGATACCAAAGGAAAAAAATGTATCTATAGAAGATTACTATGAACTA
CTATACCGAGTTTTTATAATTACAATTCACTAGAAAAGGAGCAAAAGGTTTATGAAGGG
GCTCACAGAGCGGTTGAAATTGAAGCTCTAACACCACACTCCAGCTACTGTGTAGTGGCT
GAAATATATCAGCCCATGTTAGACAGAAGAAGTCAGAGAAGTGAAGAGAGATGTGTGGAA
ATTCCATGACTTTGTGGAATTTGGCATTCAAGCAATGTGGAAATCTAAAGCTCCCTGAGAA
CAGGATGACTCGTGTGTTGAAGGATCTTATTTAAAAATTGTTTTGTATTTTCTTAAAGCAA
TATTCAGTGTACACCTTGGGGACTTCTTTGTTTATCCATTCTTTATCCTTTATATTTTC
ATTTGTAAACTATATTTGAACGACATTCCCCCGAAAAATTGAAATGTAAAGATGAGGCA
GAGAATAAAGTGTCTATGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

18/18FIGURE 18

MPKHCFLGFLISFFLTGVAGTQSTHESLKPQRVQFQSRNFHNIQWQPGRALTGNSVY
FVQYKIMFSCSMKSSHQKPSGCWQHISCNFPGCRTLAKYGQRQWKNKEDCWGTQELSCD
LTSETSDIQEPYYGRVRAASAGSYSEWSMTPRFTPWWTKIDPPVMNITQVNGSLLVIL
HAPNLPYRYQKEKNVSIEDYYELLYRVFIINNSLEKEQKVYEGAHRAVEIEALTPHSSY
CVVABIYQPMIDRRSQRSERECVEIP

Signal sequence

1-20

N-glycosylation site.

55-58

165-168

170-173

191-194

208-211

N-myristoylation site.

17-22

20-25

220-225